<Preliminary>Lignans of Chamaecyparis obtusa cv. Breviramea and Cell Suspension Cultures of Daphne odora

AUTHOR(S):

TAKAKU, Naohiro; OKUNISHI, Tomoya; MIKAME, Keigo; SUZUKI, Shiro; SAKAKIBARA, Norikazu; UMEZAWA, Toshiaki; SHIMADA, Mikio

CITATION:

TAKAKU, Naohiro ...[et al]. <Preliminary>Lignans of Chamaecyparis obtusa cv. Breviramea and Cell Suspension Cultures of Daphne odora. Wood research : bulletin of the Wood Research Institute Kyoto University 2001, 88: 44-45

ISSUE DATE:

2001-09-30

URL:

http://hdl.handle.net/2433/53139

Preliminary

Lignans of *Chamaecyparis obtusa* cv. Breviramea and Cell Suspension Cultures of *Daphne odora*[*1]

Naohiro TAKAKU[*2], Tomoya OKUNISHI[*2], Keigo MIKAME[*2], Shiro SUZUKI[*2], Norikazu SAKAKIBARA[*2], Toshiaki UMEZAWA[*2] and Mikio SHIMADA[*2]

(Received May 31, 2001)

Key words: *Chamaecyparis* obtusa cv. Breviramea, isoactifolin, *Daphne odora*, cell suspension culture

Introduction

Lignan biosynthesis has been receiving widespread interests from biochemical and stereochemical points of view.

First, a relation of lignan biosynthesis to heartwood formation is of importance, since heartwood formation is a metabolic event which is specific to woody plants, and affects significantly utilization of wood as building materials. However, little has been known about the biochemical mechanisms of heartwood formation. To address the mechanisms, elucidating biosynthetic mechanisms of lignans which are deposited specifically in heartwood would be a clue. Hinoki cypress *(Chamaecyparis obtusa)* is one of the most important building woods in Japan, and its heartwood has been known to contain significant amounts (ca. 30% of resins) of a lignan, hinokinin[1,2]. However, neither the biosynthetic mechanisms of hinokinin nor its possible precursors were known. In the present investigation, the author surveyed lignans, especially the possible precursors of hinokinin, in *C. obtusa* cv. Breviramea.

Second, stereochemical mechanisms involved in lignan biosynthesis have also been of special interests. Recent findings in the author's laboratory indicated that there are two types of stereochemical diversities in lignan biosynthesis[3–7]. One is that enantiomeric compositions of upstream lignans in the biosynthetic pathway vary significantly with plant sources, indicating a diversity of stereochemical mechanisms in the upstream steps among different plant species. The other is that of the downstream dibenzylbutyrolactone lignan. Most of this class of lignans are levorotatory having the same absolute configurations at C8 and C8′ positions, whereas those occurring in Thymelaeaceae plants are dextrorotatory. Thus, in order to access general stereochemical mechanisms of lignan biosynthesis, elucidating lignan biosynthetic mechanisms in Thymelaeaceae plants is of particular interest. This requires establishing a plant source system for biochemical studies. In this context, we have established a cell suspension culture system, which produces lignans from *Daphne odora,* a typical Thymelaeaceae plant.

1. Isolation of lignans from *Chamaecyparis obtusa* cv. Breviramea

1.1 Experimental

The young shoots with leaves of *C. obtusa* cv. Breviramea were freeze dried. The dried shoots were pulverized and extracted with hot methanol. The combined methanol extracts were suspended in distilled water, and extracted with diethyl ether. The combined diethyl ether extracts were submitted to repeated column chromatography. Each fraction obtained was submitted to repeated TLC and reversed phase HPLC to afford eleven lignans.

1.2 Results and discussion

The following eleven lignans were isolated from the young shoots with leaves of *C. obtusa* cv. Breviramea: dibenzylbutyrolactone lignans, 7-oxohinokinin (**1**), savinin (**2**), hinokinin (**3**), pluviatolide (**4**), haplomyrfolin (**5**), and

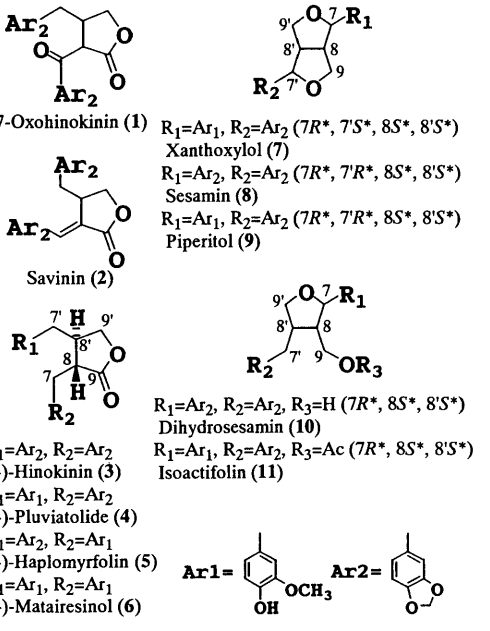

Fig. 1. Structures of lignans isolated from *Chamaecyparis obtusa* cv. Breviramea.

*1 A part of this work was presented in the 44th annual meeting of the Japan Wood Research Society, Nara, April 1994, at the 46th annual meeting of the Japan Wood Research Society, Kumamoto, April 1996, the 44th Lignin Symposium, Gifu, October 1999, and at the 51th annual meeting of the Japan Wood Research Society, Tokyo, April 2001.

*2 Laboratory of Biochemical Control.

matairesinol (**6**); furofuran lignans, xanthoxylol (**7**), sesamin (**8**), and piperitol (**9**); furan lignans, dihydrosesamin (**10**) and a new lignan (**11**) (Fig. 1).

The structure and relative configuration of **11** (Fig. 1) was determined by mass spectrometry and one- (^{1}H, ^{13}C, and NOE) and two-dimensional (^{1}H-^{1}H COSY, HMQC, and HMBC) NMR spectrometry. We proposed isoactifolin as the name of **11**. The structures of the known lignans were confirmed by comparison of their mass spectrum and NMR data with those of authentic samples and/or literature data. The eight lignans, **1**, **4**, **5**, **6**, **7**, **9**, **10**, and **11** were isolated from this plant species for the first time. Furthermore, the lignans, **3**, **4**, **5**, and **6**, were (−)-enantiomeres.

Isolation of both **4** and **5** as well as **6** suggests that the first methylenedioxy bridge formation may take place in either aromatic ring of **6** giving rise to both **4** and **5** which may be converted to **3** by the second methylenedioxy bridge formation (Fig. 3), although establishing the metabolic sequence awaits concrete evidence with biochemical experiments.

2. Lignans of cell suspension cultures of *Daphne odora*

2.1 Experimental

Callus of *Daphne odora* which has been maintained on Wolter and Skoog (WS) agar medium supplemented with 2,4-dichlorophenoxyacetic acid (1.0 μM) and 6-benzyladenine (1.0 μM) in our laboratory were used for cell suspension culture. The callus (ca. 1.5 g) was transferred to 30 ml of the same WS medium but without agar and shaken at 120 rpm under a day/night regime (16/8 hr). Following incubation for 3 weeks, the cell suspensions were filtered. The cells (ca. 2.0 g fresh weight) trapped and 10ml of the 3 week-old filtrate were transferred to a 20 ml of fresh medium in 100 ml Erlenmeyer flask. After subculturing, triplicate flasks were harvested in 4-day-intervals. The cell suspension in each flask was filtered through a filter paper, and the cells trapped were subjected to measurement of fresh weight and dry weight after feeze drying. The dried cells were grounded to powders and extracted with hot MeOH. An aliquot of the MeOH extracts was mixed with deuterium labeled lignans as internal standards, treated with β-glucosidase, and subjected to GC-MS to quantitate the lignans.

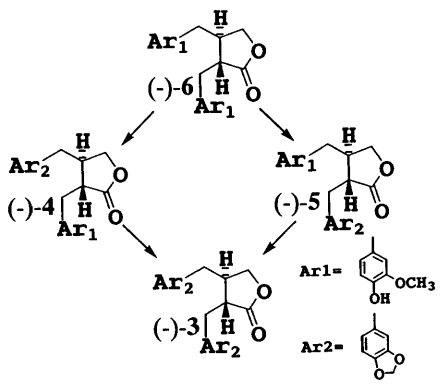

Fig. 2. A possible biosynthetic pathway for hinokinin (3) in *Chamaecyparis obtusa* cv. Breviramea.

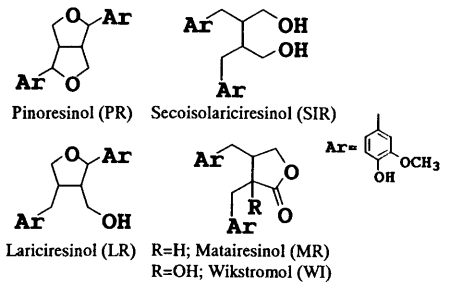

Fig. 3. Structures of lignans produced in *Daphne odora* cell suspension culture.

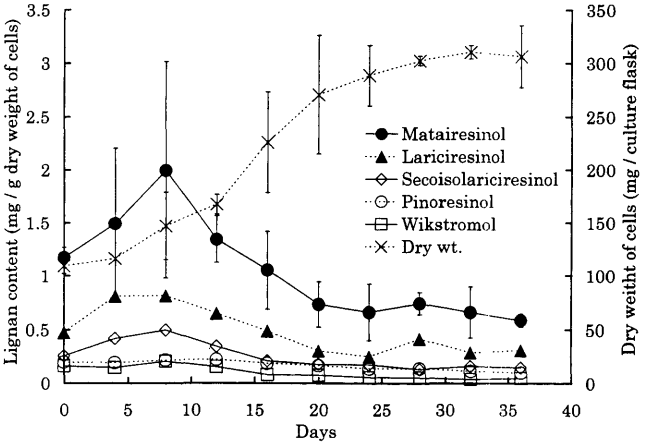

Fig. 4. Growth pattern and lignan contents in *Daphne odora* cell suspension culture.

2.2 Results and discussion

GC-MS analysis of MeOH extracts of the cells indicated the presence of the five lignans, pinoresinol (PR), lariciresinol (LR), secoisolariciresinol (SIR), matairesinol (MR), and wikstromol (WI) (Fig. 3). Growth of the cells showed a sigmoid curve with an exponential phase of 4 to 24 days followed by a stationary phase (Fig. 4). Fig. 4 showed that production of matairesinol was highest among the lignans and reached a maximum at day 8 after which levels started to decrease. The contents of the other lignans also followed similar tendency.

Thus, the cell suspension culture of *D. odora* producing lignans have been established for the first time and will be applied to further biochemical studies of lignan biosynthesis in Thymelaeaceae plants.

References

1) Y. YOSHIKI and T. ISHIGURO: *Yakugaku Zasshi*, **53**, 73–151 (1933).
2) S. KEIMATSU and T. ISHIGURO: *Yakugaku Zasshi*, **56**, 103–118 (1936).
3) T. UMEZAWA, T. OKUNISHI and M. SHIMADA: *Wood Res.*, **84**, 62–75 (1997).
4) S. SUZUKI, T. UMEZAWA and M. SHIMADA: *Biosci Biotech Biochem*, **62**, 1468–1470 (1998).
5) T. OKUNISHI, T. UMEZAWA and M. SHIMADA: *J Wood Sci*, **46**, 234–242 (2000).
6) T. OKUNISHI, T. UMEZAWA and M. SHIMADA: *J Wood Sci.*, in press (2001).
7) T. UMEZAWA: *Chemical Regulation of Plants*, in press (2001).